EFFECT OF DIFFERENT CONCENTRATIONS OF LITHIUM CARBONATE ON THIOLATE ANION IN AQUEOUS MEDIUM

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ABSTRACT

RS^- ion is called as thiolate anion and it is well stated in the literature that certain metals made complexes with thiolate anion, in order to prove this fact a study was design to check the affect of different concentrations of lithium carbonate on the status of thiolate anion by using UV-visible spectrophotometer. Results show that there is decrease in the concentration of thiolate anion with the increase in concentration of lithium ions showing the possibility of interaction between lithium ion and thiolate anion resulting in the formation of oligomeric Li-S-R.

Key words: Thiolate anion, Ellman's method (1959), Lithium Carbonate, oligomeric Li-S-R.

INTRODUCTION

During metabolism, thiolate ions are frequently generated in side cells of human body by the removal of H^+ ions from thiols specially from glutathione which is the biggest shield in human body against all types of oxidative stresses. Glutathione is very important intra cellular tripeptide which is water soluble non-protein thiol (Kromidas et al., 1990)and its importances is evident from its presence in almost all leaving organism including animals, plants, fungi etc (Zehmalek et al., 2004) and it is also found in prokaryotic cells (organisms). GSH forms the main cellular non-enzymatic antioxidant system of the human body (Halliwell, 2001).

Reduction reactions and conjugation reactions are the most important functions of glutathione and these functions are due to its –SH group which is in its cysteine unit and these reactions are the cause of removal of xenobiotic compounds and peroxides while GSH also actively takes part in regulation of cell cycles (Meister, 1992). Both normal physiological metabolism and xenobiotic conjugate with GSH and this conjugation results in depletion of GSH which is used as tool to investigate the role of GSH in antioxidant defense (Strange et al., 2000; Eaton et al., 1989). There are several known physical and chemical conditions which change intracellular concentrations of glutathione including high glucose contents (urata et al., 1996).

In 1970 and 1974, lithium was approved by USA Food and Drug Administration as treatment and maintenance therapy of mania respectively (Schsu M., 1999). Lithium can cause low blood pressure, bradycardia etc (Waring WS., 2007) and it decreases cardiace output and cardiac arrhythmias (Abdel-Zaher et al., 1999).

MATERIALS AND METHODS

Lithium Carbonate (Aldrech), Reduced glutathione i.e. GSH (Fluka), DTNB (Sigma), Double refined distilled water, potassium dihydrogen phosphate (Merck), NaoH (Sodium Hydroxide) (Fluka), hydrochloric acid Hcl 35% (Kolchlight), pH meter: model Nov: 2012 (Scientific Company Nova Ltd, Korea), Schimadzu spectrophotometer (UV-1601, Japan), oven: Memmert model U-30854 (Schwabach, Germany), Magnetic stirrer, Analytical balance model Ax 200 (Schemadzu, Japan), Beaker: 50ml, 100ml, 200ml, 500ml (pyrex Iwaki Glass, Japan), Digital micro pipette 200µl, 500 µl, 1000 µl (Scorex Swiss Finland) Were used.

PREPARATION OF STOCK SOLUTIONS

0.2 M phosphate buffer was prepared by using standard procedure. 50ml of reduced glutathione solution was prepared by taking 50ml of HcL in which 15.375mg of glutathione was added. 19.8 mg DTNB was added in 50ml phosphate buffer pH 7.6 to get 1mM DTNB solution. 2mM stock solution of lithium carbonate was prepared from which different dilution were prepared.

STANDER CURVE

2.3ml of phosphate buffer was taken in five test tubes and to each test tubes separately 0.2ml of already prepared five dilutions of GSH were added and incubated for 10minutes. To each these test tubes 0.5ml of DTNB 1mM was added and incubated for 5 minutes. After 5 minutes absorbance of each mixture was taken at 412nm. Absorbance of DTNB blank was subtracted from the absorbance of each mixture.

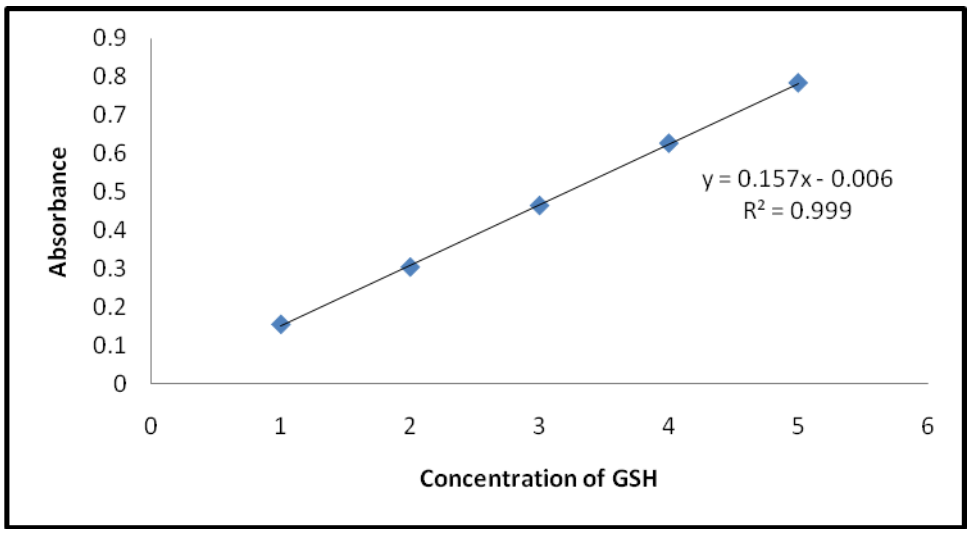

Figure 1: Standard Curve for Reduced Glutathione (GSH)

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Preparation of Mixture -1

In six test tubes, 2ml of 1mM reduce glutathione was mixed with 1ml of 1mM Elman's reagent, this mixture was mixed well and was left for 10 minutes.

Preparation of Mixture-2

2ml of each concentration (2mM to .0001mM) of lithium carbonate were added in each already prepared six test tubes containing thiolate anion. These test tubes were mixed well and were left for 10 minutes. Starting from tube containing higher concentration to lower concentration of lithium carbonate, the final concentration of lithium carbonate was 1.333mM, 0.666mM, 0.066mM, 0.0066mM, 0.00066mM and 0.000066mM respectively whereas the final concentration of glutathione in each test tube was 0.666mM.

One by one, 3ml from each tube containing mixture-2 tube were taken in sample cell and absorbance was taken at 412nm against reference cell containing 2.8ml phosphate buffer pH 7.6 and 0.2ml of 1mM GSH.

Table #1

Table No-1, Effect of different concentrations (0.0001,0.001,0.01,0.1,1,2mM)of Lithium Carbonate (LC) on the chemical Status of Thiolate Anion										
Absorbance of 5,5-Dithiobis,2-Nitrobenzoic Acid (DTNB) blank solution was 0.062 ABS at 412nm										
Concentration of GSH in final Mixture is 400 µM										
S #	**Conc. Used of Lithium Carbonate (LC)**	**Final Conc. of Lithium Carbonate (LC) in Mixture**	1st ABS	2nd ABS	3rd ABS	**Average of 3 Readings**	**Real abs*/conc. of GSH after reaction with Lithium Carbonate (LC)**		**Real abs*/conc. of GSH Blank**	
							Abs.	Conc. of GSH(µM)	Abs.	Conc. of GSH(µM)
1	0.0001mM	0.04 µM	0.609	0.603	0.600	0.604	0.542	3.49	0.765	4.91
2	0.001mM	0.4 µM	0.561	0.568	0.565	0.558	0.496	3.20	0.765	4.91
3	0.01mM	4 µM	0.530	0.527	0.525	0.527	0.465	3.00	0.765	4.91
4	0.1mM	40 µM	0.482	0.478	0.472	0.477	0.415	2.68	0.765	4.91
5	1mM	400 µM	0.399	0.395	0.393	0.396	0.334	2.17	0.765	4.91
6	2mM	800 µM	0.327	0.324	0.319	0.323	0.291	1.70	0.765	4.91
***** *Real Absorbance = Absorbance of Mixture - Absorbance of DTNB blank Solution.*										

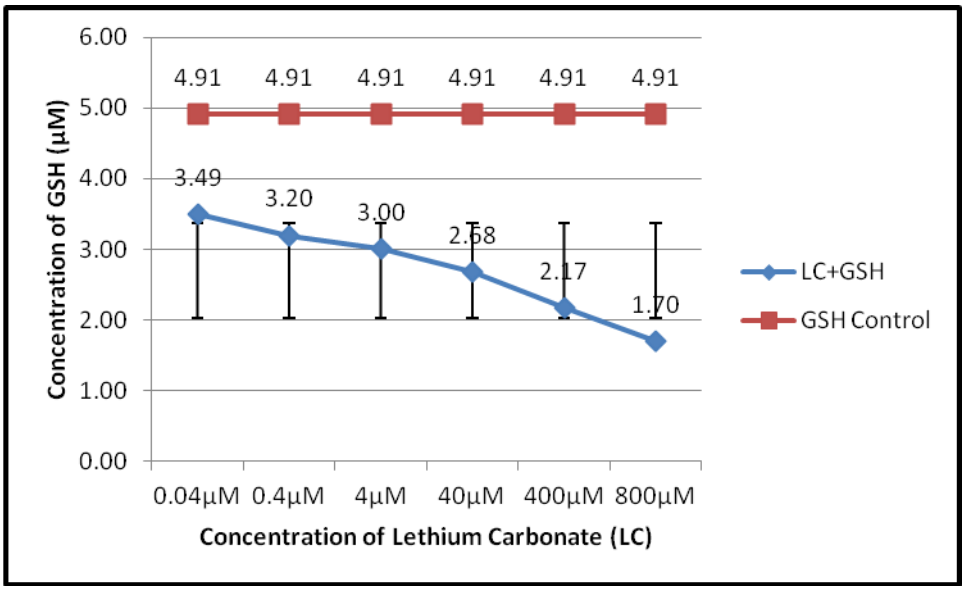

Figure 2. Effect of different concentrations (.0001mM, .001mM, .01mM, .1 mM, 1..0m M & 2.0 m M) of LC on the chemical status of thiolate anion in aqueous medium. GSH control. Results are the mean ±SE of 3 experiments.

RESULTS

AFFECT OF DIFFERENT CONCENTRATIONS OF LITHIUM CARBONATE ON THIOLATE ANION

Several dilutions (six different concentrations of lithium carbonate were used against the thiolate anion previously prepared from constant concentrations of reduced glutathione and ellman's reagent, the DTNB. As the concentration of thiolate anion was constant against each different concentration of lithium ion. The result of higher used concentration of lithium ions has decreased the thiolate anion most and the lower used concentrations of lithium ion has decreased the thiolate anion less and the last lowest used concentration of lithium carbonate against the thiolate anion the least as shown in table No.1 figure No.2.

DISCUSSION

Lithium is used as medicine since 1940 for the treatment of Mania and even today it is the drug of choice in bipolar disorder. Lithium is in use because of its antimanic, antisuicidal and anti depressant properties. Lithium is used as medicine for the treatment of many other disorders including cluster headache, shizoeffective disorder, eating disorder, impulse control disorder, attention deficit disorder, aggression, seborrheic, dermatitis, genital harps and eczematiod dermatitis etc (Jefferson.,Comprehensive textbook of psychiatry. 8th ed).

Reduce glutathione has many physiological functions and among them the major function of glutathione is to provide antioxidant defense system to the human body (Carelli et al., 1997) and this system is only effective when the concentration of reduce glutathione is high enough in intracellular as well as extracellular comportments. Each and every cell of human body is utilizing the reduced form of glutathione rapidly as in each cell glutathione is responsible to perform many physiological functions including detoxification of foreign agents like xenobiotics (Masella et al., 2005), as cofactor of many enzymes, storage and transport of cysteine (Carelli et al., 1997). Although the feedback mechanism of glutathione is present in body in which its oxidized form is converted into its reduced form but attention is needed to avoid the unnecessary and excessive use of metal complex internally or externally as the metals like lithium has great affinity to attach with reduce form of glutathione.

Our result shown in table No.1 and figure No.2 indicates that lithium which is a manovalent metal decreases the concentration of thiolate anion showing the depletion of reduced form of glutathione. We proposed that this depletion is probably due to the Li-S-R aduct formation.

PROPOSED REACTIONS

Lithium Carobate + Thiolate Anion $\longrightarrow$ Oligomeric lithium thiolate complex

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